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Notes:

THE EFFECTS OF PRESSURE ON THE BUOYANT BEHAVIOR OF
DEOXYRIBONUCLEIC ACID AND TOBACCO MOSAIC VIRUS IN A
DENSITY GRADIENT AT EQUILIBRIUM IN THE ULTRACENTRIFUGE*

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In the original analysis¹ of the behavior of macromolecules and viruses in a buoyant density gradient at equilibrium in the ultracentrifuge, all components were assumed to be incompressible. As a few hundred atmospheres are normally generated in the liquid, it is to be expected that previous results based on the assumption of incompressibility require re-examination.

Several consequences arise on consideration of previously ignored pressure-dependent terms. It is shown below that these may be separately considered.

- (a) There is a redistribution of solute with respect to solvent in the binary medium.
- (b) The solution is compressed with no change in molality in each of the thin layers perpendicular to the centrifugal field in the liquid column. This compression adds a *compression density gradient* to the *composition density gradient*.
- (c) The banding macromolecular species is compressed and moves to a new neutrally buoyant solution. A change in band shape occurs.

It is shown in the analysis below that changes in salt molality in response to pressure are small and may be neglected. The combined effects of compressing the solution and the macromolecular species are significant and affect the band shape, band position, and buoyant density.

Theory.—Although the effects of pressure in two- and multicomponent sedimentation equilibrium experiments²⁻⁴ have been considered by previous workers, the problem is examined here with special reference to the formation of the density gradient and the behavior of neutrally buoyant macromolecules. For a two-component system at equilibrium in a centrifugal field at constant temperature, the thermodynamic relation,

$$M_2(1 - \bar{v}_{2,P}\rho)\omega^2r\,dr = \left(\frac{\partial\mu_2}{\partial m_2}\right)_P dm_2, \quad (1)$$

is valid.⁵ This expression remains valid for free solute⁶ in the three-component system at low polymer concentration. In equation (1), M_2 is the molecular weight, $\bar{v}_{2,P}$ the anhydrous partial specific volume, $\mu_2(P, m_2)$ the chemical potential, and m_2 the molality of the solute. The density of the solution, the radial distance, and the angular velocity are $\rho(P, m_2)$, r , and ω , respectively. In equation (1) \bar{v}_2 , $(\partial\mu_2/\partial m_2)$, and ρ are pressure-dependent variables.

The first two variables are expressed in terms of first-order expansions in pressure about a pressure of 1 atm by Taylor's theorem. Higher-order terms in these expansions are small and therefore neglected. Throughout this paper P will refer to the pressure above atmospheric pressure.

$$\left(\frac{\partial \mu_2}{\partial m_2}\right)_P = \left(\frac{\partial \mu_2}{\partial m_2}\right)_{P=0} + \left(\frac{\partial^2 \mu_2}{\partial P \partial m_2}\right)P.$$

Substituting the relation $(\partial \mu_2 / \partial P)_{m_2} = M_2 \bar{v}_2$ into the above equation gives a relation in terms of experimentally accessible variables,

$$\left(\frac{\partial \mu_2}{\partial m_2}\right)_P = \left(\frac{\partial \mu_2}{\partial m_2}\right)_{P=0} + M_2 \left(\frac{\partial \bar{v}_2}{\partial m_2}\right)_{P=0} P. \quad (2)$$

Similarly,

$$\bar{v}_2, P = \bar{v}_2, P=0 + \left(\frac{\partial \bar{v}_2}{\partial P}\right)_{P=0} P.$$

Introducing the relation for the partial specific isothermal compressibility of the solute, $\kappa_2 \equiv -[1/\bar{v}_2(d\bar{v}_2/dP)]_{P=0, m_2}$

$$\bar{v}_2, P = \bar{v}_2, P=0(1 - \kappa_2 P). \quad (3)$$

By a similar Taylor's expansion, the density of the solution may be expressed in terms of the isothermal compressibility coefficient of the solution, κ , at $P = 0$, and the density, $\rho^0(m_2)$,⁷ of the solution of molality, m_2 , at atmospheric pressure.

$$\rho = \rho^0/(1 - \kappa P) \quad (4)$$

Upon substitution of the effects of pressure, equations (2), (3), and (4), the differential equation (1) becomes

$$\frac{dm_2}{dr} = \frac{M_2 \left[1 - \frac{(1 - \kappa_2 P)}{(1 - \kappa P)} \bar{v}_2^0 \rho^0\right] \omega^2 r}{\left(\frac{\partial \mu_2}{\partial m_2}\right)^0 + M_2 \left(\frac{\partial \bar{v}_2}{\partial m_2}\right)^0 P}. \quad (5)$$

Equation (5) provides a means of calculating the composition density gradient,

$$\left(\frac{d\rho}{dr}\right)^0 = \frac{dm_2}{dr} \left(\frac{d\rho}{dm_2}\right)^0 = \left(\frac{d\rho}{dm_2}\right)^0 \frac{M_2 \left[1 - \frac{(1 - \kappa_2 P)}{(1 - \kappa P)} \bar{v}_2^0 \rho^0\right] \omega^2 r}{\left(\frac{\partial \mu_2}{\partial m_2}\right)^0 + M_2 \left(\frac{\partial \bar{v}_2}{\partial m_2}\right)^0 P}, \quad (6)$$

and by comparison with the comparable equation at atmospheric pressure,

$$\left(\frac{d\rho}{dr}\right)^0 = \left(\frac{d\rho}{dm_2}\right)^0 \frac{M_2 [1 - \bar{v}_2^0 \rho^0] \omega^2 r}{(\partial \mu_2 / \partial m_2)^0} \equiv \frac{\omega^2 r}{\beta^0}, \quad (7)$$

we can estimate the effect of pressure on salt distribution. Equation (7) defines β^0 , a parameter previously calculated by Ifft, Voet, and Vinograd⁸ and designated by them as β . Because the pressure correction terms are small, equation (6) may be simplified by expansion, retaining only first-order terms in the corrections. Incorporating the definition of β^0 expressed by equation (7),

$$\left(\frac{d\rho}{dr}\right)^0 = \frac{\omega^2 r}{\beta^0} \left\{ 1 - \left[\frac{\bar{v}_2^0 \rho^0 (\kappa - \kappa_2)}{1 - \bar{v}_2^0 \rho^0} + \frac{M_2 \left(\frac{\partial \bar{v}_2}{\partial m_2}\right)^0}{\left(\frac{\partial \mu_2}{\partial m_2}\right)^0} \right] P \right\} \quad (8)$$

$$\left(\frac{d\rho}{dr}\right)^0 \equiv \frac{\omega^2 r}{\beta^0} \{1 - \phi P\}.$$

The quantity ϕ has been evaluated for CsCl solutions, Table 1. Pohl's compressibility data,⁹ and data for ρ^0 and \bar{v}_2^0 used in reference 8 were employed in the calculations.¹⁰

TABLE 1
PRESSURE CORRECTION TERMS FOR THE COMPOSITION DENSITY GRADIENT, CsCl 25°C

ρ^0	$\kappa \text{ atm}^{-1},$ $\times 10^6$	$\kappa_2 \text{ atm}^{-1},$ $\times 10^6$	$\bar{v}_2^0 \rho^0$	$M_2 \left(\frac{\partial \bar{v}_2}{\partial m_2}\right)^0 / \left(\frac{\partial \mu_2}{\partial m_2}\right)^0$ $\times 10^6$	$\phi \text{ atm}^{-1},$ $\times 10^6$
1.3	34.2	-33.4	0.342	42.4	77.5
1.5	29.5	-15.9	0.405	26.8	57.7
1.7	25.8	-5.9	0.464	21.8	49.2

As the pressure at the center of a 1.1 cm CsCl column, $\rho^0 = 1.7 \text{ gm cm}^{-3}$, at 44,770 rpm is approximately 130 atm,¹¹ the ϕP correction terms will generally be less than 0.01 and are therefore neglected. The corrections become smaller if shorter CsCl columns are used. The advantage of neglecting the pressure correction to the salt distribution is that ρ^0 may then be calculated with the equation,

$$\rho^0 = \rho_e^0 + \int_{r_e}^r \left(\frac{d\rho}{dr}\right)^0 dr = \rho_e^0 + \int_{r_e}^r \frac{\omega^2 r}{\beta^0} dr, \quad (9)$$

where r_e is the radius at which the molality of CsCl is that of the initial homogeneous solution, and ρ_e^0 is the initial density at atmospheric pressure. The iso-concentration distance, r_e , has been determined⁸ for different salts as a function of ρ_e^0 and ω^2 . Since the effect of pressure on salt redistribution is small, these data remain valid.

Having established that significant salt redistribution is not caused by the pressures encountered during density gradient analyses, we obtain the *physical density gradient* by differentiating equation (4) with respect to r ,

$$\frac{d\rho}{dr} = \frac{1}{1 - \kappa P} \left(\frac{d\rho}{dr}\right)^0 + \frac{\rho^0(m)}{(1 - \kappa P)^2} \frac{d(\kappa P)}{dr}. \quad (10)$$

Introducing $dP/dr = \rho \omega^2 r$, equation (10) for the case of constant compressibility coefficient becomes

$$\frac{d\rho}{dr} = \frac{1}{1 - \kappa P} \left[\left(\frac{d\rho}{dr}\right)^0 + \frac{\kappa \rho^0 \omega^2 r}{(1 - \kappa P)^2} \right]. \quad (11)$$

Neglecting pressure-correction terms of the order of 1% of $d\rho/dr$, the above equation is

$$\frac{d\rho}{dr} = \left[\left(\frac{1}{\beta^0}\right) + \kappa \rho^0 \right] \omega^2 r \equiv \frac{\omega^2 r}{\beta}. \quad (12)$$

The second term in equation (12) is the significant compression term and is tabulated with $1/\beta^0$ in Table 2 for CsCl solutions. The values for the compressibility coefficient κ are interpolated from the data of Pohl.⁹ His data are extrapolated linearly from $\rho = 1.4$ to $\rho = 1.8$ g cm⁻³.

With the aid of equation (12) it has been demonstrated¹² in the case that \bar{v}_s is a function only of solute activity and pressure that the buoyancy condition in a density gradient experiment is¹³

$$\rho_0^0 = \frac{1}{\bar{v}_{s,0}^0} \left\{ 1 - \frac{(\kappa - \kappa_s)P_0}{1 - \left(\frac{\partial \rho_0^0}{\partial a_1^0} \right)_P \left(\frac{da_1^0}{d\rho^0} \right)} \right\} \quad (13)$$

The slope of the ρ_0^0 versus P_0 relation is $-\psi/\bar{v}_{s,0}^0$, where ψ is $(\kappa - \kappa_s)/[1 - (\partial \rho_0^0/\partial a_1^0)_P (da_1^0/d\rho^0)]$. In the following experiments, a study of the dependence of ρ_0^0 on pressure is made. This dependence is found to be linear.

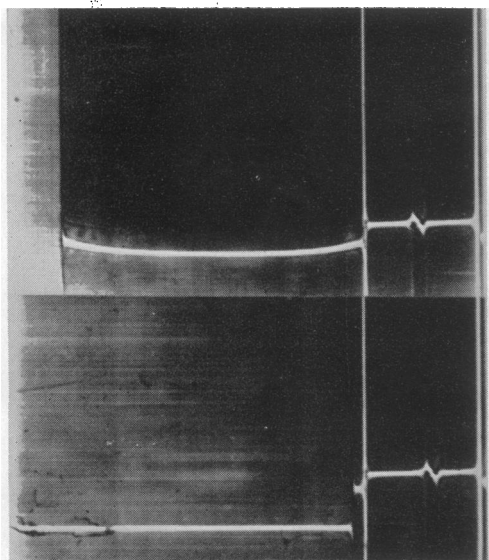


FIG. 1.—The effect of pressure on band position of T-4 DNA in CsCl solution $\rho_s^0 = 1.699$ g. cm.⁻³ at 25°C at 44,770 rpm. The pressure at band center in the lower part of the photograph is 54.5 atm. and in the upper part is 170.7 atm.

The Determination of ψ .—The value of ψ may be measured with adequate accuracy by noting the displacements of equilibrium bands upon changing the pressure. This was accomplished in two ways: An immiscible oil was layered in successive increments on a short column of CsCl solution containing a buoyant macromolecular species and rotated after each increment until equilibrium was attained. The cell containing a band was rotated to equilibrium at varying angular velocities.

Experimental.—Materials: The CsCl was obtained from Maywood Chemical Co., Maywood, N. J., and recrystallized three times. Emission spectroscopic analyses performed in the Department of Geology showed less than 0.03% metallic impurities. The silicone oil was Dow Corning 550 fluid, Lot No. 88-161. According to the manufacturer, it is a methyl- and phenyl-substituted polysiloxane containing less than 10 parts per million of metal salt impurity. The FC-43 fluoro-chemical

was obtained from the Minnesota Mining and Manufacturing Co. and is stated to be triperfluorobutylamine. The tobacco mosaic virus strain U 1 (TMV) was kindly supplied by Professor S. Wildman, University of California at Los Angeles. The stock solution contained 5.47 mg/ml TMV in 0.001 versene, pH 7.5. The preparation of the T-4 bacteriophage DNA has been described.¹⁴ All other chemicals used were reagent grade materials.

Procedure.—The effect of pressure on band position: The oil column experiments were performed with both TMV and DNA solutions. The speed-variation experiments were performed only with DNA solutions. The solutions delivered into the standard 4°, 12-mm, Kel-F center-piece cell assembly had densities, ρ^0 , of 1.325 and 1.704 g cm.⁻³ for TMV and DNA respectively. The first solution contained 46 μ g cm.⁻³ of TMV and was buffered with 0.01 M tris at pH 7.0. The second solution was buffered with 0.02 M tris at pH 9. The DNA concentration in terms of $E_{280}^{1\text{cm}}$ was 0.085. All experiments were begun by filling cells with 0.02 ml of fluorocarbon, 0.18

ml of CsCl solution, and 0.02 ml of silicone oil. Both the fluorocarbon and the silicone oil were included to provide accurate means of recording the top and bottom menisci of the CsCl column. This column was about one-fourth the length attainable in the standard centerpiece.

The solutions were centrifuged at 25.0°C. and photographed with the schlieren optical system after equilibrium had been established. The actual speeds were evaluated from odometer readings made at the beginning and end of the run. These agreed with the nominal values within $\pm 0.02\%$. In these short columns at 44,770 rpm, the DNA runs required 14 hours and the TMV runs about 90 minutes to reach equilibrium. A typical pair of exposures is shown in Figure 1. The top and bottom CsCl menisci and the silicone oil meniscus were taken to be the centers of the symmetrical meniscus images. These, as well as the counter balance reference edges and the positive and negative peaks, associated with the inflections in the polymer concentration distributions, were

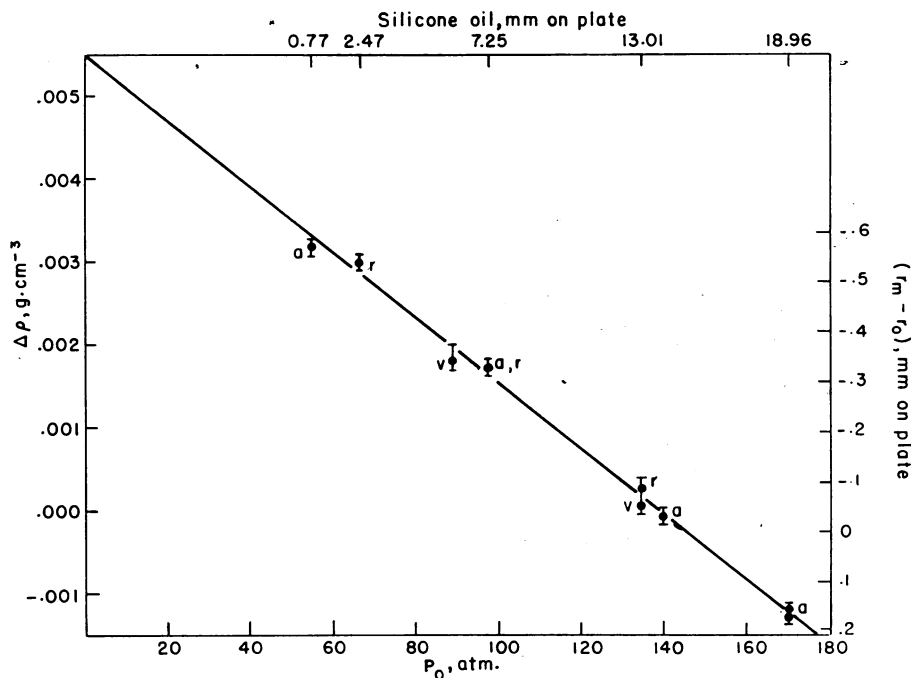


FIG. 2.—Buoyant density increments for T-4 DNA in CsCl solution at various pressures, $\rho_e^0 = 1.699 \text{ g. cm.}^{-3}$ at 25°C at 44,770 rpm. Expt. no. 1. a, addition of oil; r, removal of oil; v, points obtained at 31,410 rpm and 39,460 rpm. The coordinates at the top and right-hand side indicate the original data. Slope = $-3.94 \times 10^{-5} \text{ g. cm.}^{-3}/\text{atm}$. The maximum error intervals are indicated.

measured with a coordinate plate and film Comparator Model M 2001-P, Gaertner Scientific Co. Chicago, Illinois. Reading accuracy was $\pm 0.01 \text{ mm}$, which corresponds to ± 5 microns in the cell. The band position was taken to be the average of the distances associated with the positive and negative peaks.

In the oil column experiments, the cells were reopened at the filling hole after the first equilibration and 0.16 ml silicone oil added. The solutions were again run to equilibrium. The procedure was repeated twice again. Silicone oil was similarly removed with a 24-gauge needle in two or three stages.

In one of the DNA runs, after the cell was filled with silicone oil and centrifuged to equilibrium, the angular speed was dropped to 31,410 rpm, then raised to 39,460 rpm, and again raised to 44,770 rpm. Thirty-five hours were required for equilibrium at 39,460 rpm and 65 hours at 31,410 rpm. The slow step in these experiments was band motion. The band width became constant after 22 hours at both speeds. After 5 days continuous running, the band returned to its original position at 44,770 rpm as indicated by the double point at 170 atm (Fig. 2).

The density of the silicone oil at 25°C. was measured in a calibrated 0.3 ml micropipet. The density was $1.067 \pm 0.001 \text{ g cm}^{-3}$.

The Effect of Pressure on the Composition Density Gradient.—In the theoretical part of this paper, it was noted that the composition density gradient is insensitive to pressure. To check this conclusion, the effect of pressure on the refractive index gradient was measured. The 4° Kel-F, 12-mm standard cell was filled with 0.02 ml of fluorocarbon, 0.34 ml of CsCl solution, $\rho^0 = 1.339 \text{ gm cm}^{-3}$ and 0.02 ml of silicone oil. The rotor was brought to 44,770 rpm as rapidly as possible, and schlieren images at a bar angle of 55° were photographed immediately upon reaching this speed and again after 10 hours, at equilibrium. The experiment was repeated after thorough homogenizing of the CsCl solution and the addition of 0.34 ml of silicone oil. The composition density gradient was obtained at r_e from the difference in elevations between the early and equilibrium exposures.

For these experiments, tracings were made of 20-fold cell to vellum enlargements with an Omega D-2 enlarger. The CsCl meniscus was used for lateral orientation and the image of a horizontal wire mounted just in front of the schlieren camera for vertical orientation. Elevations proportional to the compression density gradient were measured with an accuracy of 0.4 per cent.

Calculations.—For evaluation of the results in accordance with equation (13), P_0 , the pressure at band center, and ρ_0^0 , the buoyant density at atmospheric pressure, must be derived from the experimental data. The pressure is the sum of the pressures generated by the action of the field in the silicone oil and in CsCl solution over the band.

The pressure at any point in a liquid volume is obtained by integration of the equation

$$\frac{dP}{dr} = \rho \omega^2 r. \quad (14)$$

In order to integrate this expression, the r dependence of ρ is needed.

In a solution,

$$\rho = \rho_\alpha + \int_{r_\alpha}^r \frac{\omega^2 r}{\beta} dr, \quad (15)$$

where r_α is an arbitrary reference. It is sufficiently accurate to assume that β is a constant.

$$\rho = \rho_\alpha + \frac{\omega^2}{\beta} \left(\frac{r^2 - r_\alpha^2}{2} \right) \quad (16)$$

Substituting this expression into equation (1) and integrating from r_1 to r_2 ,

$$P_2 - P_1 = \rho_\alpha \omega^2 \left(\frac{r_2^2 - r_1^2}{2} \right) + \frac{\omega^4}{2\beta} \left[\frac{(r_2^4 - r_1^4)}{4} - r_\alpha^2 \frac{(r_2^2 - r_1^2)}{2} \right]. \quad (17)$$

To simplify computation, r_α is so selected that the term in the brackets in equation (17) vanishes. For this condition, r_α is the root mean square between r_1 and r_2 . In all cases the use of the arithmetic mean for r_α did not introduce a significant error.

$$r_\alpha = \sqrt{\frac{r_2^2 + r_1^2}{2}} = \frac{r_2 + r_1}{2} + \frac{1}{8} \frac{(r_2 - r_1)^2}{r_2}$$

The pressure at band center caused by the CsCl solution was evaluated as follows: The quantity r_α is the root mean square radius between band center, r_0 , and the CsCl meniscus, r_{cm} . The density ρ_α is obtained with sufficient accuracy with equation (9), assuming β^0 constant, the limits of integration being r_e and r_α . This neglects the effect of pressure on ρ_α . Substitution into equation (5) yields

$$P_{r_0, \text{CsCl}} = \frac{\rho_\alpha \omega^2}{1.013 \times 10^6} \frac{(r_0^2 - r_{cm}^2)}{2} \quad (18)$$

The numerical coefficient is introduced in order to express pressure in atmospheres. ρ_α was found to vary only 0.12% in a given series over the entire range of oil column lengths and was therefore considered constant.

In the oil column treated as a one-component system, $1/\beta^0 = 0$, and $1/\beta = \kappa' \rho^2$, equation (12), where κ' is the isothermal compressibility of the oil. The same arguments used for the CsCl column apply here. In this case, however, the compressibility and the pressure contribution of the oil are larger; the effect of pressure on ρ_α is therefore taken into account. For a good estimate of the pressure P_α , equation (14) is integrated between the oil meniscus, r_{om} , and r_α , under the assumption that the density is constant.

$$P_\alpha = \frac{\rho^0 \omega^2}{1.013 \times 10^6} \frac{(r_\alpha^2 - r_{om}^2)}{2} = \frac{\rho^0 \omega^2}{1.013 \times 10^6} \frac{(r_{cm}^2 - r_{om}^2)}{4} \quad (19)$$

The r_α was eliminated with the definition of r_α . Upon substituting $\rho_\alpha = \rho^0(1 + \kappa' P_\alpha)$ and equation (19) into equation (17), and letting r_2 be the cesium chloride meniscus, r_{cm} , and r_1 be the oil meniscus, r_{om} ,

$$P_{cm} = \rho^0 \left\{ 1 + \kappa' \left[\frac{\rho^0 \omega^2}{1.013 \times 10^6} \frac{(r_{cm}^2 - r_{om}^2)}{4} \right] \right\} \left(\frac{(r_{cm}^2 - r_{om}^2)}{2} \right). \quad (20)$$

The pressure at band center is the sum of the pressures given by equations (18) and (20).

Now the shifts in band position must be expressed in density units. The buoyant density at atmospheric pressure, ρ_0^0 , is calculated with equation (9). In order to express the dependence of β^0 on r , β^0 is expanded:

$$\beta^0 = \beta_\gamma^0 + \left(\frac{d\beta^0}{dr} \right)_\gamma (r - r_\gamma)$$

where r_γ is again an arbitrary reference radius. Integrating and evaluating r_γ so that the $(d\beta^0/dr)_\gamma$ term equals zero as before, the following equations are obtained,

$$r_\gamma = \frac{r_2 + r_1}{2} + \frac{(r_2 - r_1)^2}{12 r_2} \dots$$

and

$$\rho_2^0 - \rho_1^0 = \frac{\omega^2}{\beta_\gamma^0} \left(\frac{r_2^2 - r_1^2}{2} \right).$$

As in the previous case, the selection of β^0 at the arithmetic mean rather than at r_γ will not introduce a significant error.

Since the density shifts due to the pressure variation are very small, the quantity $\Delta\rho = \rho_0^0 - \rho_e^0$ was evaluated and not ρ_0^0 itself. The desired expression is

$$\Delta\rho = \rho_0^0 - \rho_e^0 = \frac{\omega^2}{\beta_e^0} \left(\frac{r_0^2 - r_e^2}{2} \right). \quad (21)$$

The following relation utilizing the original comparator data (capital R 's) and the magnification factor, MF , was used,

$$(r_0^2 - r_e^2) = \frac{2 \times 5.725}{MF} \left(R_0 - \frac{R_{cm} + R_{fm}}{2} \right) + \frac{1}{(MF)^2} \left(R_0^2 - \frac{R_{cm}^2 + R_{fm}^2}{2} \right), \quad (22)$$

where R_{fm} is the radius of the fluorocarbon meniscus.

According to theory, a plot of $\Delta\rho$ versus P_0 should yield a straight line of slope $-\psi/\bar{v}_{s,0}^0$ and intercept $(1/\bar{v}_{s,0}^0 - \rho_e^0)$. Figures 2 and 3 present the data obtained for one series each of DNA and TMV. The value of $\bar{v}_{s,0}^0$ was determined from the intercept. From the intercept and the slope, values for ψ were obtained. Duplicate experiments in each case were performed.

In the measurements, the radii were located with a precision of 0.01 mm on the plate. The values of P_0 were calculated with no significant error. The values of $\Delta\rho$ are very sensitive to the accuracy of measurement because these values are derived from the difference of the squares of two numbers of comparable magnitudes. The maximum error in each of the $\Delta\rho$ values was

calculated to be $\pm 0.00012 \text{ gm cm}^{-3}$. The two points obtained at lower speeds show an asymmetric maximum-error interval. This is due to the difficulty in measuring the position of the broader bands at lower speeds. The flare at each maximum had to be used to find the peak. It was known from observations of the relation of the flare to the actual maximum on photographs at 44,770 rpm that the flare from both peaks is displaced approximately 0.02 mm toward the center of rotation. This corresponds to an error of $-0.00010 \text{ gm cm}^{-3}$.

The probable error of the function $\Delta\rho$ was computed using an expression given in Margenau and Murphy.¹⁵ Assuming a probable error of 0.005 mm in each comparator measurement, a probable error for the function, $\Delta\rho$, was found to be $0.00003 \text{ gm cm}^{-3}$.

The adherence of the points after the oil removal to the straight line showed that no detectable evaporation occurred. The points in general can be determined with an accuracy of $0.0001 \text{ gm cm}^{-3}$. This corresponds to an evaporative loss of 0.026 mg of H_2O and a meniscus shift of 0.5μ . Because the meniscus can be located with an accuracy of only 10μ , it is clear that the band position is a much more sensitive test for evaporation than the change in meniscus height. A density shift of 0.004 gm cm^{-3} is required to cause an observable shift in CsCl column length.

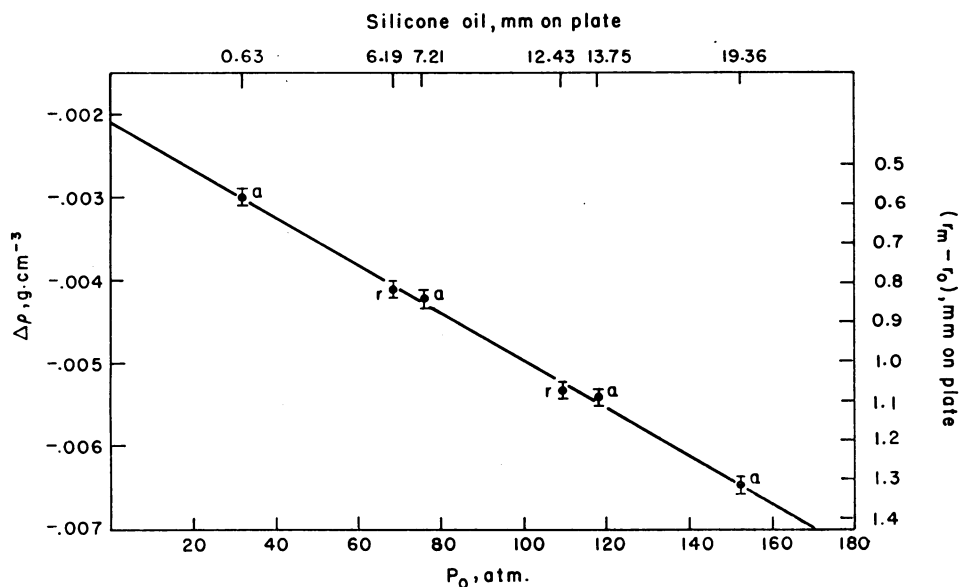


FIG. 3.—Buoyant density increments for TMV in CsCl solution at various pressures, $\rho_e^\circ = 1.324 \text{ g. cm}^{-3}$ at 25°C at 44,770 rpm. Expt. no. 3. *a*, addition of oil; *r*, removal of oil. The coordinates at the top and right-hand side indicate the original data. Slope = $-2.88 \times 10^{-5} \text{ g. cm}^{-3}/\text{atm}$. The maximum error intervals are indicated.

Results and Discussion.—The examination of the effects of pressure-dependent terms in equilibrium sedimentation of macromolecules in a density gradient illuminates two important interrelated problems, solvation and compression. These effects are also encountered in conventional sedimentation equilibrium but are smaller and frequently neglected.

The effects of compression on the physical density gradient in the CsCl solutions have been analyzed. The result, Table 2, is to increase the physical density gradient by 8.5–10.8%. These effects are independent of speed. At a density of 1.7 gm cm^{-3} , the compression gradient is 9% of the composition gradient.

That the compression gradient may be simply added to the composition gradient is shown in experiments in which the refractive index gradient at equilibrium was

TABLE 2
THE EFFECT OF COMPRESSION ON THE PHYSICAL DENSITY GRADIENT IN CsCl SOLUTIONS AT
EQUILIBRIUM IN THE ULTRACENTRIFUGE AT 25°C

ρ^0	$1/\beta^0 \times 10^{10}$	$\kappa\rho^{02} \times 10^{10}$	$\frac{1/\beta^0 + \kappa\rho^{02}}{1/\beta^0} = \frac{1/\beta}{1/\beta^0}$
1.2	5.042	0.543	1.108
1.3	6.468	0.586	1.091
1.4	7.429	0.629	1.085
1.5	8.034	0.673	1.084
1.6	8.351	0.714	1.085
1.7	8.400	0.757	1.090
1.8	8.231	0.797	1.097

evaluated in a short CsCl column with and without a layer of silicone oil. The effect on the net refractive index gradient at the root mean square position in the cell due to this layer of oil, which corresponded to a pressure increment of 72.1 atmospheres, was -1.3% . This result is within the experimental measuring errors and the effect predicted by the theory, Table 1. It is consistent with the previously obtained agreement⁸ between the composition density gradient obtained optically at the pressure in the cell and calculated from physical chemical data obtained at atmospheric pressure. Three approaches now show that the CsCl-water distribution is not significantly changed by compression, even though the solution is compressed and the physical density gradient increased.

In density gradient experiments the macromolecular species does not respond only to the physical density gradient. Of importance is the *effective density gradient*, which is shown in the preceding paper¹² to be

$$\left(\frac{d\rho}{dr}\right)_{\text{eff}} = \left[\frac{1}{\beta^0} + \psi\rho^{02}\right] (1 - \alpha)\omega^2 r \quad (23)$$

where

$$\alpha = \left(\frac{\partial\rho_0^0}{\partial a_1^0}\right)_P \left(\frac{da_1^0}{d\rho^0}\right).$$

The quantity ψ must therefore be known in order to evaluate correctly the effective density gradient and the molecular weight of either the anhydrous or the hydrated species.

The values of ψ and $\bar{v}_{s,0}^0$ obtained from the oil column experiments are given in Table 3. The numbers in the fifth column, $1/\bar{v}_{s,0}^0$, are the values of the composition variable ρ_0^0 when the band is at atmospheric pressure. The values listed as ρ_0^0 are values calculated for the pressure at the middle of a 1.1 cm column of CsCl solution at 44,770 rpm. Thus significant deviations in ρ_0^0 will be encountered by workers banding the same material at different radii in the cell.

If proper experimental data are available, ψ can be used to calculate the apparent compressibility of the solvated polymer. A more complete discussion of this matter

TABLE 3
EFFECT OF PRESSURE ON BUOYANT MOLECULES IN A DENSITY GRADIENT

Material	Expt. No.	$\psi \times 10^6$ atm ⁻¹	$\frac{1}{\bar{v}_{s,0}^0}$	$\frac{1}{\bar{v}_{s,0}^0}$	ρ^0
T-4 Bacteriophage	1	23.1	0.587	1.704	1.699
DNA	2	23.5	0.587	1.703	1.698
Tobacco mosaic	3	21.8	0.756	1.322	1.319
virus, strain U 1	4	21.5	0.757	1.321	1.318

is presented in the preceding article.¹⁶ If the effects of solvation are neglected, the value of κ_s for *TMV* obtained from ψ and from Pohl's compressibility data is $12.9 \times 10^{-6} \text{ atm}^{-1}$. This value agrees favorably with the value $10 \times 10^{-6} \text{ atm}^{-1}$ which Jacobson¹⁷ obtained for various proteins in dilute salt solutions. The agreement may be the result of coincidence, because the effects of solvation have been neglected.

For *T-4 DNA* ψ was found to be $23.3 \times 10^{-6} \text{ atm}^{-1}$. The parameter α for *DNA* in *CsCl* is 0.24.¹⁶ These numbers may now be used to calculate the difference in compressibility, $\kappa - \kappa_s = 17.7 \times 10^{-6} \text{ atm}^{-1}$. From this difference κ_s is found to equal $8.7 \times 10^{-6} \text{ atm}^{-1}$. It should be stressed that κ_s is an apparent compressibility. It not only includes the compression of the solvated species, but also a pressure dependence of the amount of hydrated water.

The use of an insoluble marker material, such as a thin film of plastic, has been suggested by Szybalski.¹⁸ Solvation effects for such a material should be negligible

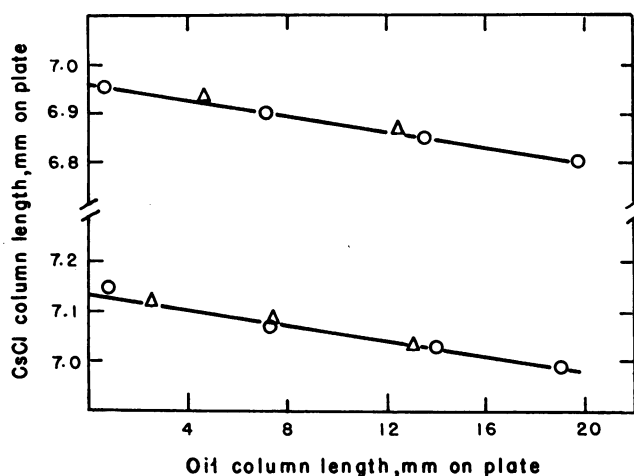


FIG. 4.—The effect of pressure on *CsCl* solution column length. O, addition of oil; Δ , removal of oil. The lower line is from expt. no. 1 and the upper line is from expt. no. 2.

because surface areas are relatively small. Large pressure effects on marker positions are to be expected and have been observed.¹⁸

DNA samples, denatured, isotopically substituted, or of differing composition, can be used as density markers, providing that the dependence of ψ and α on pressure for the marker is the same as the sample under investigation. The effective density gradient, equation (23), is used to calculate density differences in such experiments.

Cell Distortion.—The *CsCl* column length was found to change in a characteristic manner with the level of the supernatant silicone oil (Fig. 4). The two sets of points correspond to two independent experiments. In these, the cell was disassembled and reassembled with a solution of slightly different density. The linear and elastic response reflects the behavior of a cell assembly with a Kel-F centerpiece and quartz windows. The observed decrease in *CsCl* column length of 2.1% is 10.8 times larger than that attributable to the compression of the solution.

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³ Williams, J. W., K. E. Van Holde, R. L. Baldwin, and H. Fujita, *Chem. Revs.*, **58**, 715 (1958).

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⁶ Refer to reference 12 for the definition of the term "free solute."

⁷ The superscript zero is used throughout this paper to designate variables at atmospheric pressure. The density $\rho^0(m_2)$ is a function only of m_2 and should be thought of as a composition variable. The actual density ρ is a measure of physical density and is a function of m_2 and pressure.

⁸ Ifft, J. B., D. H. Voet, and J. Vinograd, *J. Phys. Chem.* (in press).

⁹ Pohl, F., Dissertation, Rheinische Friedrich Wilhelms Universität, Bonn, Germany (1906).

¹⁰ κ_2 was evaluated with the aid of the following equation derived from equation (4) and with the aid of the intercept method for determining partial specific quantities:

$$\kappa_2 = \kappa + \frac{1}{\rho^0 \bar{v}_2^0} (\kappa_1 - \kappa)$$

The quantity κ_i is the intercept at $Z_2 = 1$ of the tangent to the κ versus weight fraction of CsCl' Z_2 curve.

¹¹ All subsequent error approximations are based on a pressure of 130 atmospheres.

¹² Hearst, J. E., and J. Vinograd, these PROCEEDINGS, **47**, 999 (1961).

¹³ Equation (13) is equation (22) in reference 12. The subscript s refers to the solvated species; a_1^0 is the activity of the solute at atmospheric pressure.

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¹⁵ Margenau, H., and G. M. Murphy, *The Mathematics of Physics and Chemistry* (Princeton: D. Van Nostrand Co., 1956), p. 515.

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AMBIVALENT *rII* MUTANTS OF PHAGE T4

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The *rII* mutants of phage T4 differ from the standard type in being inactive on strains of *Escherichia coli* lysogenic for phage λ . While the mutants attach to the cells and inject their DNA, progeny fail to appear. In this paper it is shown that one or another subset of the *rII* mutants can become active as a result of modification of the host, either by mutation or by the action of 5-fluorouracil. The effects are characterized by extreme specificity, applying only to certain *rII* mutations at certain points within the two *rII* cistrons. The phenomenon resembles what has been called, in many other systems, "allele-specific suppression,"¹ in which a muta-